Leucine-Rich Repeat Transmembrane Proteins Instruct Discrete Dendrite Targeting in an Olfactory Map

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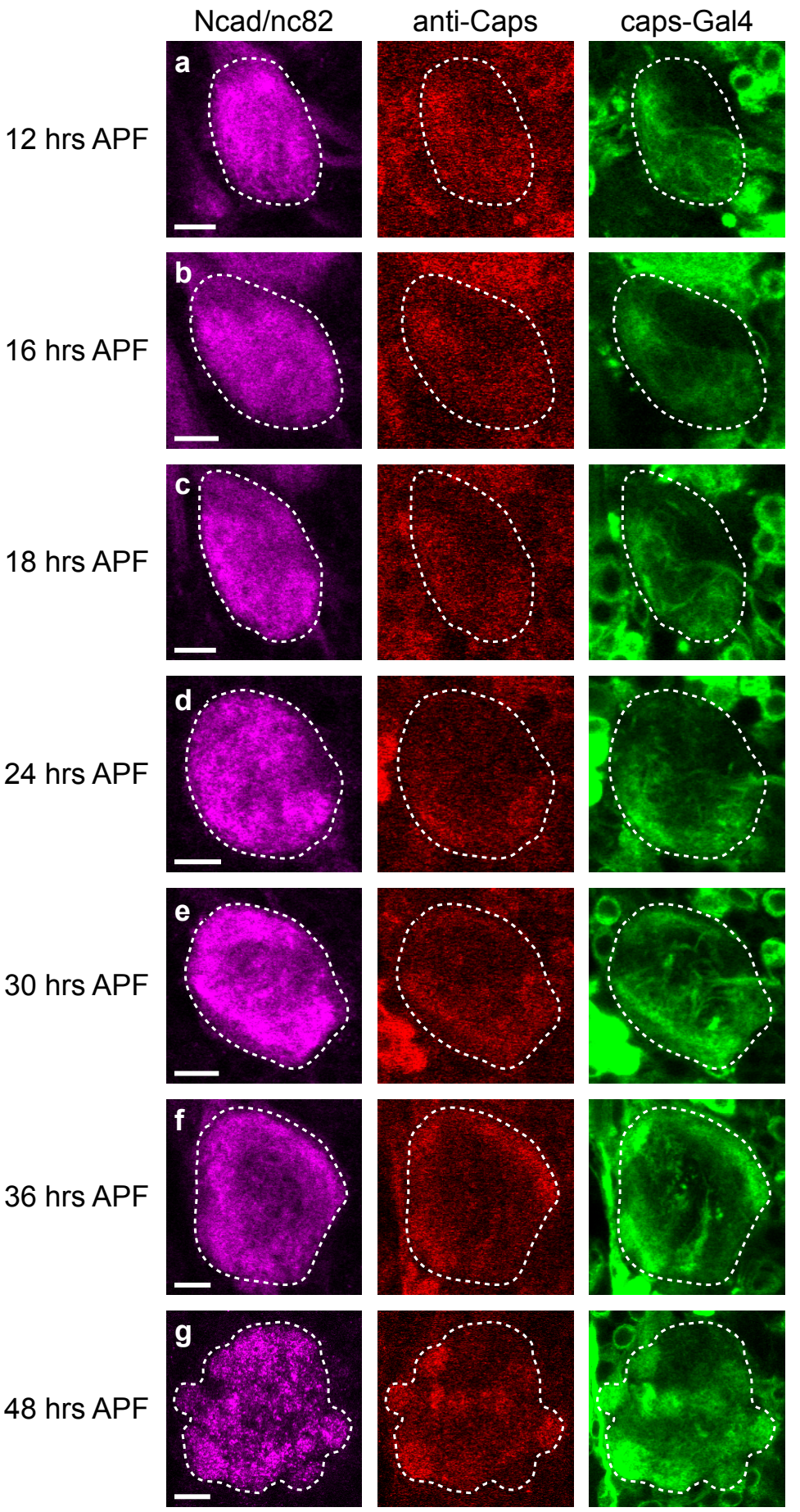
Supplementary Figure 1



Supplementary Figure 1. Time course analysis of Caps expression during development.
(**a-g**) The developing antennal lobes were stained from 12 h APF to 48 h APF by antibodies against Caps (central panels) and *caps-Gal4*-driven mCD8-GFP (right panels). Neuropil is visualized in the left panels by N-cadherin (**a-f**, from 12 h APF to 36 h APF) or nc82 (**g**, 48 h APF). Caps protein is present in the developing antenna lobe throughout the period when PN dendrites are making their target decisions, and it is not distributed evenly as compared to the neuropil markers. Scale bars represent 10 μm. All images are single confocal sections.

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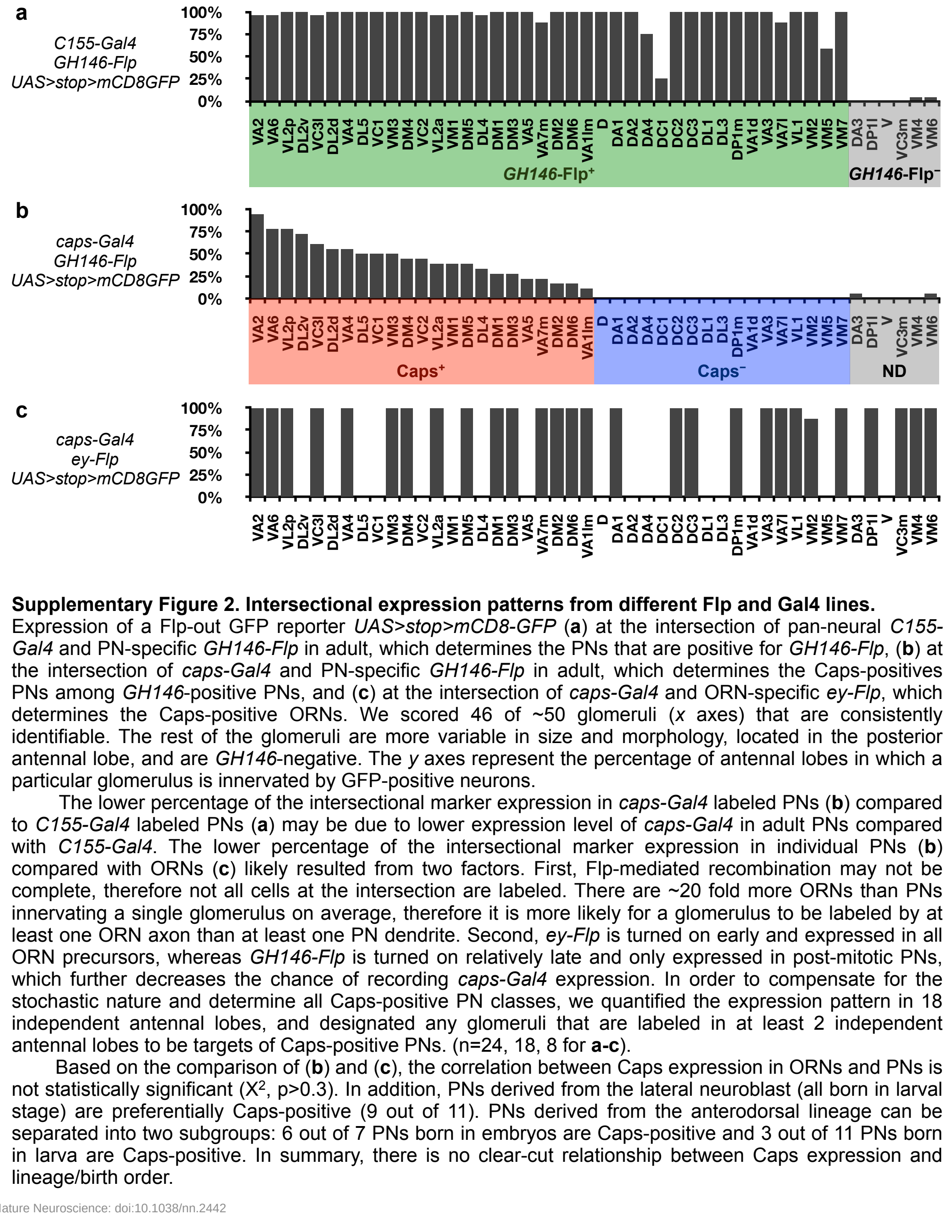
Supplementary Figure 2



Supplementary Figure 2. Intersectional expression patterns from different Flp and Gal4 lines.
Expression of a Flp-out GFP reporter *UAS>stop>mCD8-GFP* (**a**) at the intersection of pan-neural *C155-Gal4* and PN-specific *GH146-Flp* in adult, which determines the PNs that are positive for *GH146-Flp*, (**b**) at the intersection of *caps-Gal4* and PN-specific *GH146-Flp* in adult, which determines the Caps-positives PNs among *GH146*-positive PNs, and (**c**) at the intersection of *caps-Gal4* and ORN-specific *ey-Flp*, which determines the Caps-positive ORNs. We scored 46 of ~50 glomeruli (*x* axes) that are consistently identifiable. The rest of the glomeruli are more variable in size and morphology, located in the posterior antennal lobe, and are *GH146*-negative. The *y* axes represent the percentage of antennal lobes in which a particular glomerulus is innervated by GFP-positive neurons.

The lower percentage of the intersectional marker expression in *caps-Gal4* labeled PNs (**b**) compared to *C155-Gal4* labeled PNs (**a**) may be due to lower expression level of *caps-Gal4* in adult PNs compared with *C155-Gal4*. The lower percentage of the intersectional marker expression in individual PNs (**b**) compared with ORNs (**c**) likely resulted from two factors. First, Flp-mediated recombination may not be complete, therefore not all cells at the intersection are labeled. There are ~20 fold more ORNs than PNs innervating a single glomerulus on average, therefore it is more likely for a glomerulus to be labeled by at least one ORN axon than at least one PN dendrite. Second, *ey-Flp* is turned on early and expressed in all ORN precursors, whereas *GH146-Flp* is turned on relatively late and only expressed in post-mitotic PNs, which further decreases the chance of recording *caps-Gal4* expression. In order to compensate for the stochastic nature and determine all Caps-positive PN classes, we quantified the expression pattern in 18 independent antennal lobes, and designated any glomeruli that are labeled in at least 2 independent antennal lobes to be targets of Caps-positive PNs. (n=24, 18, 8 for **a-c**).

Based on the comparison of (**b**) and (**c**), the correlation between Caps expression in ORNs and PNs is not statistically significant (X^2, $p>0.3$). In addition, PNs derived from the lateral neuroblast (all born in larval stage) are preferentially Caps-positive (9 out of 11). PNs derived from the anterodorsal lineage can be separated into two subgroups: 6 out of 7 PNs born in embryos are Caps-positive and 3 out of 11 PNs born in larva are Caps-positive. In summary, there is no clear-cut relationship between Caps expression and lineage/birth order.

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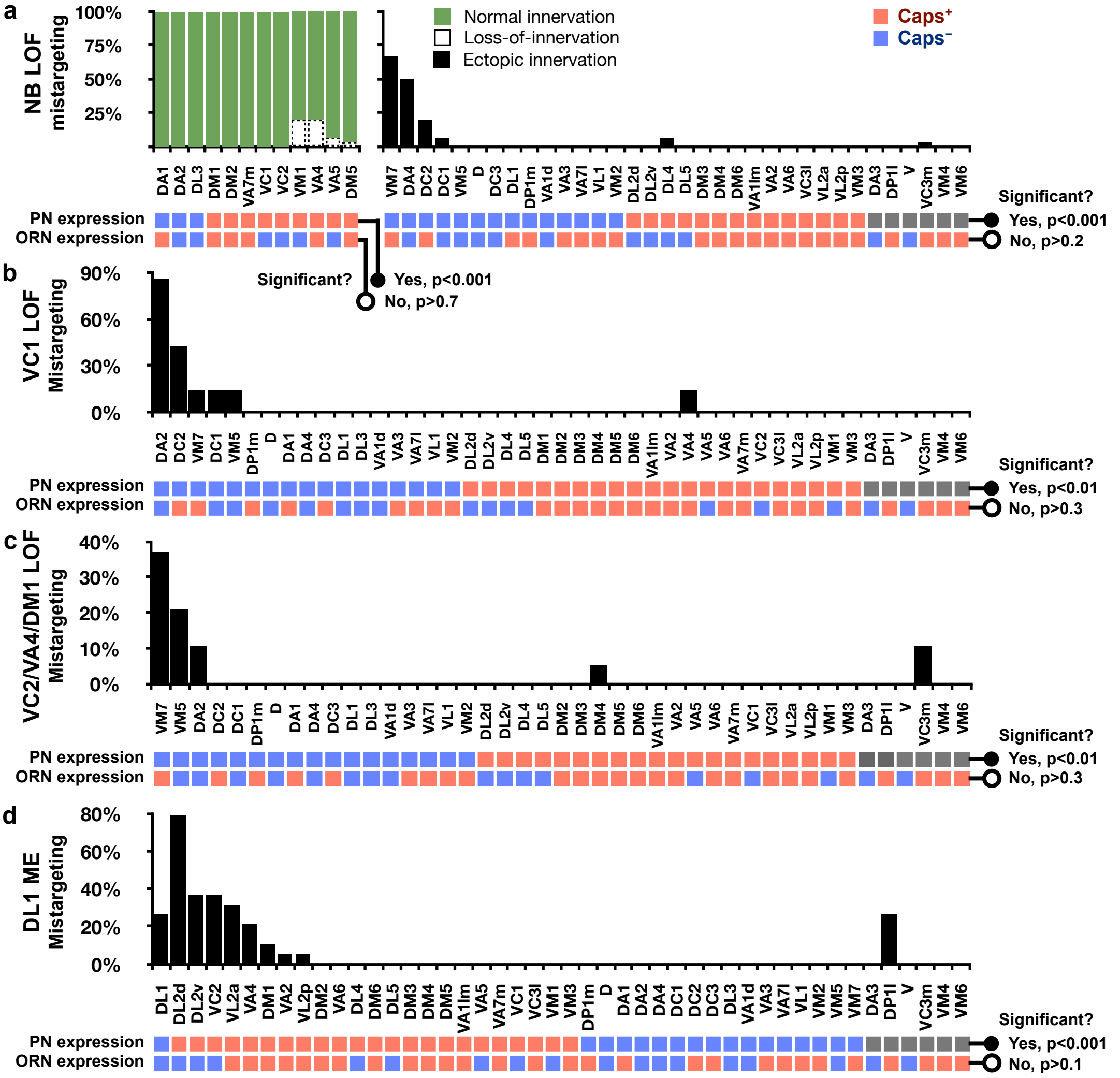
Hong et al, 2009

Supplementary Figure 3



Supplementary Figure 3. Statistics of mistargeting biases with regard to Caps expression in PNs or ORNs. Quantification of glomerular innervation pattern in *caps*$^{-/-}$ neuroblast clones (**a**), *caps*$^{-/-}$ single cell clones (**b-c**) and Caps misexpression in DL1 single cell clones (**d**) were taken from **Figure 2d, 3t, 3v** and **4j**. We added Caps expression information below individual classes of PNs and ORNs (orange, Caps-positive; blue, Caps-negative). We determined whether the preference of mistargeting events that occur in glomerular targets of Caps-positive or Caps-negative PNs (or ORNs) is statistically significant, and showed the corresponding p-values to the right of Caps expression pattern. In each case, there is a significant correlation between where mistargeting occurs and Caps expression pattern in PNs, but no significant correlation between where mistargeting occurs and Caps expression pattern in ORNs. In addition, there is no clear-cut relationship between ectopic targets and the lineage/birth order of corresponding PNs.

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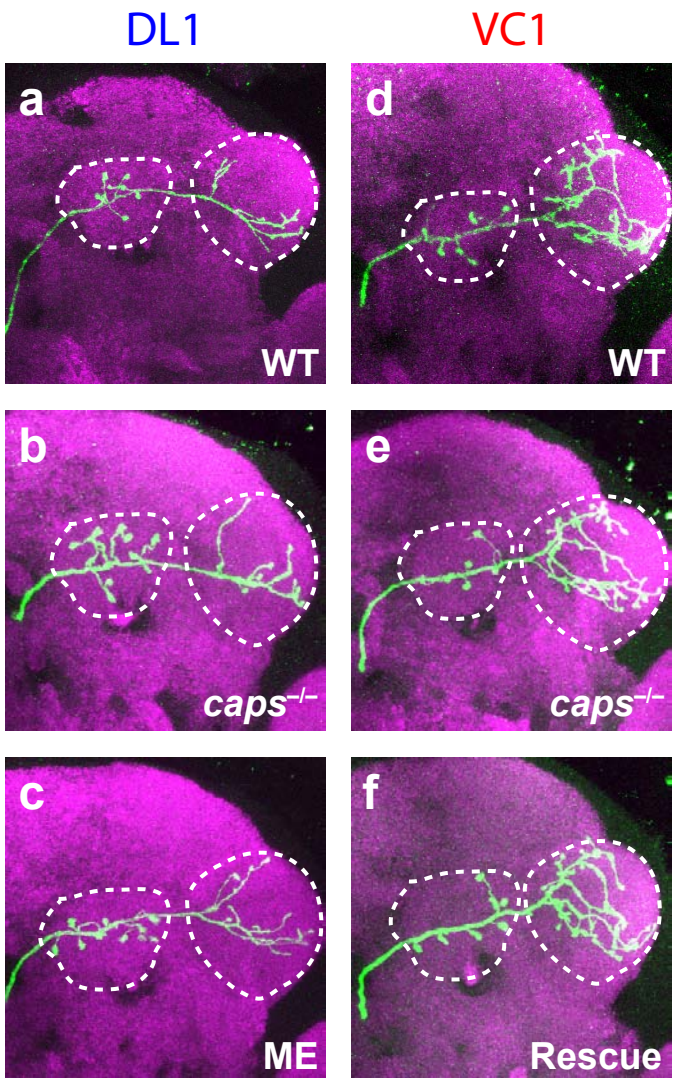
Supplementary Figure 4



Supplementary Figure 4. Normal axon targeting of PNs in *caps* mutant and misexpression. Axon targeting to the mushroom body calyx and lateral horn (outlined) of single cell DL1 clones (**a-c**) and VC1 clones (**d-f**) are shown for three genotypes as indicated. Caps misexpression in Caps-negative DL1 PNs or Caps loss in Caps-positive VC1 PNs did not change the class-specific lateral horn arborization patterns. See Methods for class identification. DL1 wild-type, n=10; DL1 *caps*$^{-/-}$, n=10; DL1 misexpression, n=10; VC1 wild-type, n=10; VC1 *caps*$^{-/-}$, n=7; VC1 rescue, n=6.

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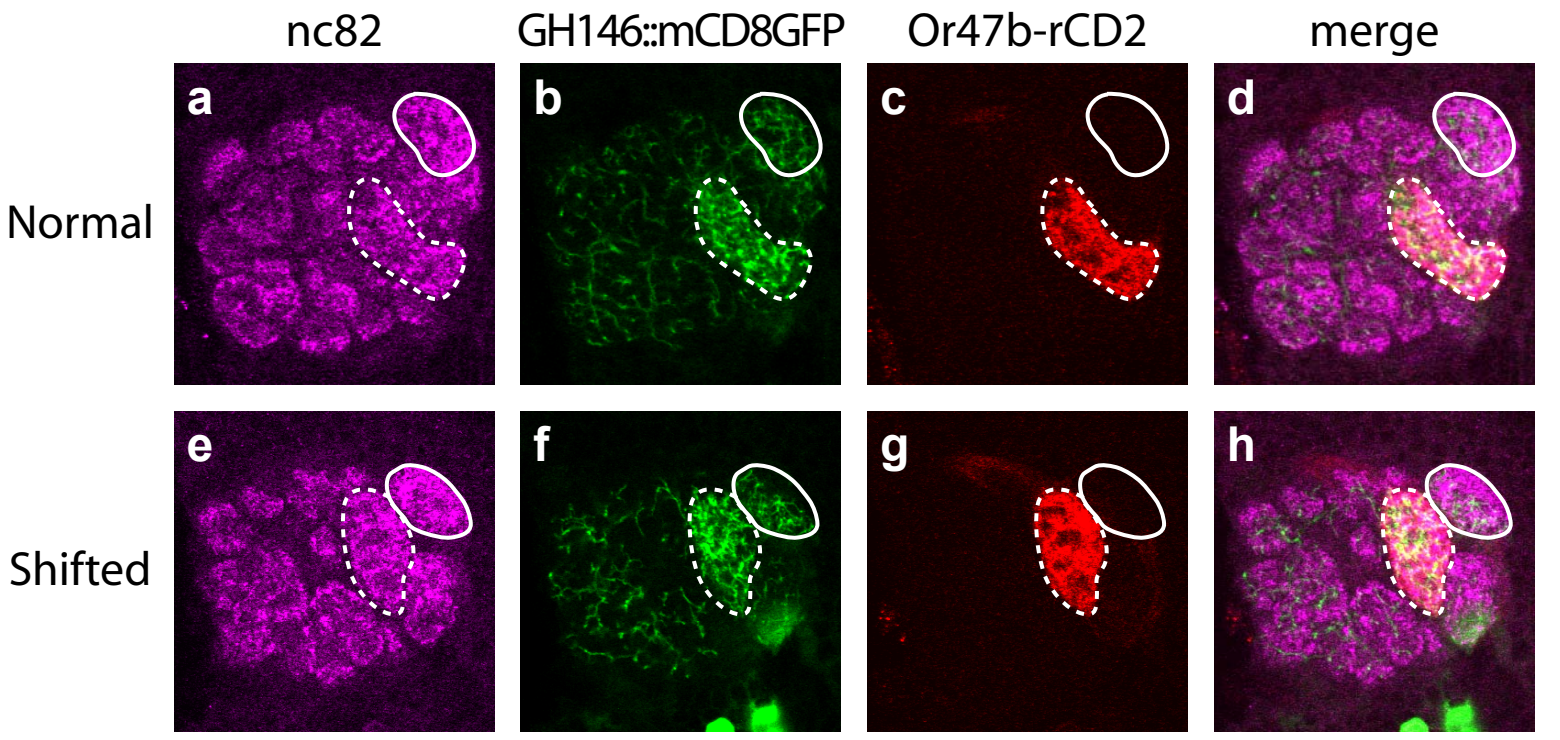
Supplementary Figure 5



Supplementary Figure 5. Loss of Caps in PNs does not disrupt the proper targeting of ORN axons. Normal *GH146-Gal4* labeled ventral neuroblast MARCM clones consist of uniglomerular PNs that target dendrites to the DA1, VA1lm, and VL1 glomeruli, and a pan-antennal-lobe-projecting PN[1]. In the anterior section of the antennal lobe, PN dendrites targeting to DA1 (solid outline) and VA1lm (dashed outline) can be distinguished above the background of the less dense dendrites of the pan-antennal-lobe-projecting PN (top panel, second from left). DA1 and VA1lm are separated by the VA1d glomerulus. However, in 4 out of 13 $caps^{-/-}$ ventral neuroblast clones, we found a dorsomedial shift of dendrites targeting to the VA1lm glomerulus, such that it extensively borders the DA1 glomerulus (an example is shown in the bottom panel). We additionally labeled ORNs that normally target to the VA1lm glomerulus with a transgene *Or47b-rCD2* (ref. 2), and found that in all 4 cases Or47b axons shift correspondingly, such that the synaptic matching between ventral VA1lm PN and Or47b axon is not disrupted, despite the loss of Caps in ventral VA1lm PNs. Panels from left to right: nc82 staining that labels all glomeruli; MARCM labeling of ventral neuroblast clones by *mCD8-GFP*; *Or47b-rCD2* labeling of Or47b ORN axons; merge of the three channels. Genotype: $hsFlp^{122}$ *UAS-mCD8-GFP; GH146-Gal4 UAS-mCD8-GFP / Or47b-rCD2;* $caps^{c28fs}$ *FRT2A / G80 FRT2A*.

References:

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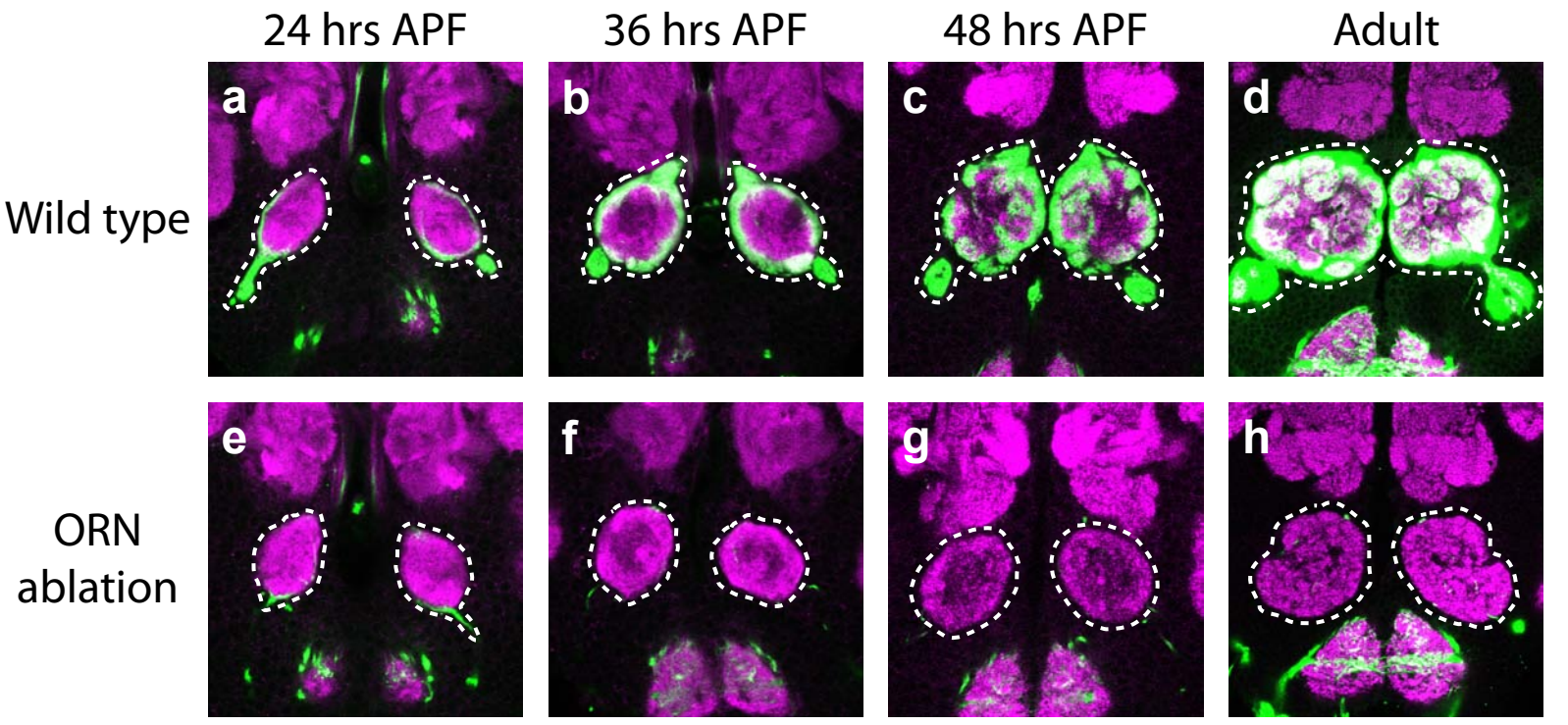
2. Berdnik, D., Chihara, T., Couto, A. & Luo, L. Wiring stability of the adult Drosophila olfactory circuit after lesion. *J. Neurosci.* **26**, 3367-3376 (2006).

Supplementary Figure 6



Supplementary Figure 6. Efficacy of pan-ORN ablation during development. (**a-d**) *Pebbled-Gal4* is expressed in all ORNs during and after ORN axons arrive at the antennal lobe. (**e-h**) *Pebbled-Gal4* and *ey-Flp* are utilized to drive the expression of a flip-out toxin *RicinA* in the intersectional region of *Pebbled-Gal4* and *ey-Flp*. This strategy ablates almost all ORNs before their axons enter the developing antennal lobe. Compared with normal ORNs (**a-d**), most of the ablated ORNs die or fail to send axons to the antennal lobe and few ORN axons arrive at the edge of the antennal lobe at 24 h APF (**e**). These remaining axons do not enter the antennal lobe and eventually die (**f-h**). Antennal lobes and ORN entry bundles are outlined by dotted lines. *Pebbled-Gal4* also labels some central neurons, notably in the suboesophageal ganglia (ventral to the antennal lobes), which are not ablated because these neurons do not express *ey-Flp*. All images are single confocal sections. Genotype: *Pebbled-Gal4, ey-Flp; UAS-mCD8-GFP; UAS>stop>RicinA / UAS-caps*.

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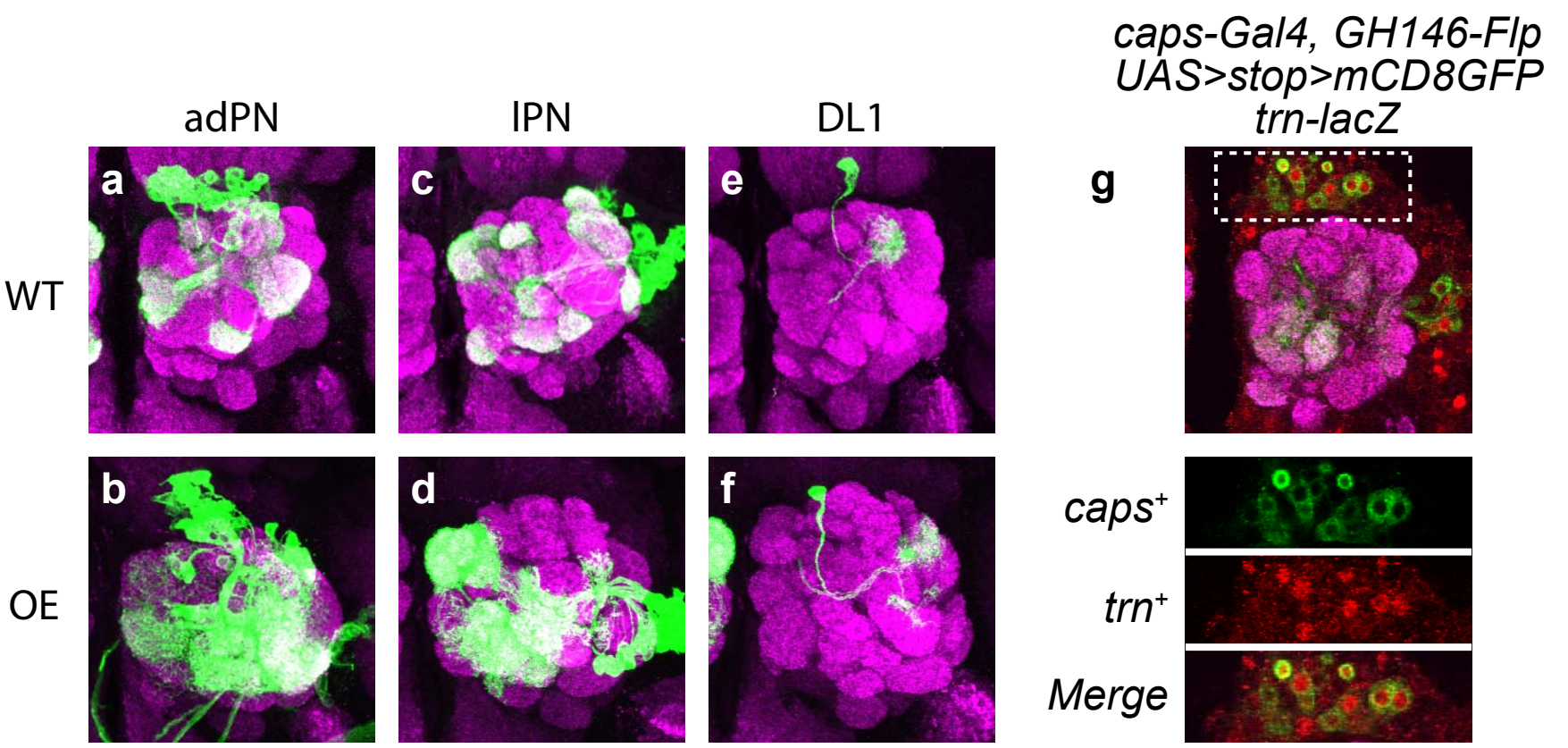
Supplementary Figure 7



Supplementary Figure 7. Trn overexpression phenotypes and overlapping expression with Caps in PNs. (**a-f**) Dendrite targeting of wild-type and Trn-overexpressing PNs in neuroblast or single-cell MARCM clones. Overexpression of Trn (*P{GS6}10885*) in neuroblast clones (**b,d**) or DL1 single cell clones (**f**) causes a strong mistargeting of dendrites to other glomeruli compared to wild-type controls (**a,c,e**). (**g**) Expression of *trn-lacZ* (red) together with *caps-Gal4*, *GH146-Flp*, *UAS>stop>mCD8-GFP* (green). A magnified view of the cell bodies is shown in the lower panels.

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